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HYPERTENSION, CARDIOVASCULAR RISK AND POLYMORPHISMS IN GENES CONTROLLING THE CYTOCHROME P450 PATHWAY OF ARACHIDONIC ACID: A SEX-SPECIFIC RELATION?

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Abstract

Hypertension is a multifactorial disease in which the interplay of genetic and environmental factors that maintain blood pressure stable throughout life is altered. Cytochrome P450 (CYP)-derived metabolites of arachidonic acid such as epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE), active on vascular tone, endothelial function and renal sodium reapportion, have been identified as candidate mediators in the development of hypertension in several animal models, with remarkable sex-specific effect. Several SNPs, some recognized as functional, in human genes implicated in EETs/20-HETE biosynthesis and metabolism, such as *CYP2J2* and *CYP4A11,* have been tested for association with blood pressure, hypertension and its long-term cardiovascular consequences in different populations, with conflicting results. A sex-specific effect, related to *CYP4F2* polymorphisms and expression, has been observed in association studies. This finding indicates that altered 20-HETE bioactivity underlay the excess of hypertension and associated vascular events observed in men with respect to women and is consistent with the results from experimental models. Further epidemiological and mechanistic studies are required to confirm the effect of lipid mediators on blood pressure in humans and define the mechanisms of a putative sex-specific effect.

Key Words

Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, genetic polymorphism, hypertension, sex, cytochrome P450

Introduction

Hypertension and its long term cardiovascular consequences are major determinants of morbidity and mortality worldwide[1-3]. Both genetic and environmental factors are implicated in the homeostasis of blood pressure and the development of hypertension[4;5]. Despite large heritability of these traits, only few genes have been unequivocally associated to blood pressure phenotype, with common single nucleotide polymorphisms (SNPs) conferring individual risk of a very limited increase in mean blood pressure (a few mmHg) and rare variants responsible for Mendelian forms of hypertension or hypotension having a larger effect on blood pressure, but a negligible prevalence in the general population[6;7]. Also the Genome Wide Association Studies (GWAS) identified only a minority of genetic loci and candidate genes potentially associated with blood pressure and the development of hypertension[8-10]. The notion that the prevalence of hypertension and cardiovascular disease differ between men and women is well established, being women protected from cardiovascular events until menopause, with a rapid increase in their risk profile beyond that age[11-13]. Responsibility for the observed differences has been attributed to sex hormones, although hormone replacement therapy failed to decrease coronary events in post-menopausal women and had negligible effect on blood pressure[14-17]. Gene-specific effects have been indicated as responsible, but most of genetic studies did not include sex as a variable in data analysis and a recent meta-analysis pooling thousands of subjects of previous GWASs did not identify any sex-specific effect of genes associated to blood pressure/hypertension[18]. Despite these drawbacks, the evidence that sex-related differences exist in cardiovascular risk is strong; the challenge is to find out which genes are actually implicated and though which pathway they act.

The cytochrome P450 (CYP) is a complex enzyme system in which different isoforms share significant sequence homology and act on myriad of endogenous and exogenous substrates, representing one of the major systems implicated in drugs metabolism. The CYP enzymatic system may have a role in the development of hypertension as well as cardio- and cerebrovascular events[19;20], being implicated in steroidogenesis, including androgens and estrogens biosynthesis and metabolism[21]. Arachidonic acid is oxidized by the CYP mono-oxygenase to produce hydroxy- and epoxy-arachidonic acid derivatives with vasoactive and natriuretic properties: namely 20-hydroxyeicosatetraenoic acid (20-HETE) and 5,6-, 8,9-, 11,12- and 14,15 epoxyeicosatrienoic acids (EETs), which are metabolized to the correspondent dihydroxyeicosatrienoic acids (DHETs), mostly inactive compounds, by soluble Epoxide Hydrolase (sEH). Thus, CYP isoforms, by producing vasoactive and natriuretic compounds, and interacting with sex hormones, could represent a cross-road in sex-related risk of cardiovascular disease.

In the present review, we describe the available evidences linking specific CYPs involved in 20-HETE and EETs metabolism, along with *EPHX2*, the gene codifying for sEH, with the development of hypertension and discuss possible sex-specific effects in the light of the results from experimental, mechanistic, genetic and epidemiological studies.

CYP450 isoforms, 20-HETE and EETs formation and action.

CYP2C/CYP2J and CYP4A/CYP4F subfamilies are the predominant and functionally relevant vascular and renal arachidonic acid epoxygenases and ω-hydroxylases, in humans, catalyzing the production of EETs and 20-HETE[19;20]. EETs bioactivity is terminated by the conversion, catalyzed by sEH, into the corresponding DHETs, almost inactive compounds.

20-HETE and EETs play critical roles in the regulation of renal, pulmonary, and cardiac function and vascular tone. In particular, EETs hyperpolarize vascular smooth muscle cells by increasing the open-state probability of the calcium-activated potassium (K_{Ca}) channels, whereas 20-HETE exerts opposite effects[19;20]. The vasoconstrictors angiotensin II, vasopressin, and norepinephrine, activate phospholipases in vascular smooth muscle cells (VSMCs) and increase the release of arachidonic acid, thus triggering the formation of 20-HETE[19;20]. CYP-derived metabolites of arachidonic acid play an important role also in the modulation of renal reabsorbtion of sodium: acting on different channels at tubular level, both 20-HETE and EETs exert natriuretic effects. [19;20] Thus, by acting at vascular level, EETs and 20-HETE exert antihypertensive and pro-hypertensive effects, respectively, whereas both compounds have antihypertensive properties through their effects at tubular level[19;20].

Beside vasoactive properties, EETs also display potent anti-inflammatory, antiplatelet and antithrombotic activities[22]. EETs increase the rate of growth in endothelial cells, stimulate angiogenesis and inhibit the proliferation of human VSMCs. Therefore, EETs have been proposed to have a dual vasoprotective effect by promoting neovascularisation in ischemic tissues and by inhibiting atherosclerosis[23]. EETs have been reported to be cardioprotective by virtue of their anti-inflammatory activity and their capacity of modulating several cardiac ion channels. 20-HETE has been described as harmful having pro-inflammatory and pro-oxidative effects, that lead to damage of vascular endothelium[20].

Studies using animal models to explore the patophysological role of 20-HETE and EETs

A role in altered sodium handling and control of vascular tone has been attributed to altered 20-HETE and EETs bioactivity in animal models of hypertension. These include DOCA salt hypertensive rats, Lyon rats, spontaneously hypertensive rats (SHR), Dahl Salt sensitive (Dahl S) rats and two models of hypertension induced by the intravenous infusion of angiotensin II and by constructing a transgenic rats with two functionally active renin genes. Increased biosynthesis of 20-HETE has been recognized to be responsible for the increased reactivity to constrictor agonists in the renal vasculature of SHR, whereas decreased generation of 20-HETE in the renal tubuli may contribute to the shift of the pressure-natriuresis relation, responsible for salt-sensitive hypertension in Dahl S rats[19;24;25].

Interestingly, the results of several studies on different rat strains, using knockout models or specific pharmacological tools to alter the CYPs system, show a clear dimorphism between male and female animals: only male animals have an increased susceptibility to hypertension and either castration or the administration of androgen inhibitors revert the hypertensive phenotype[26-31].

Sex specificity in animal models

Male mice knockout for the *CYP4A14* gene develop androgen-dependent hypertension which is attenuated by castration and restored by androgen replacement[26]. Treatment of normotensive rats with 5alpha-dihydrotestosterone (DHT) is associated with an increase in systolic blood pressure both in males and females[27;29]. The ratio of 20-HETE to EETs in renal interlobar arteries from male rats is twofold higher than from female rats. Moreover, DHT treatment eliminates any difference between males and females in the 20-HETE to EETs ratio[29]. The effect of DHT administration on blood pressure is accompanied by the up-regulation of CYP isoforms responsible for the synthesis of 20-HETE (CYP4A12 in mice, CYP4A8 in rats) in the renal vasculature and down-regulation of those responsible for the synthesis of EETs (CYP2C23)[26;29]. In DHT-induced hypertension a selective CYP4A inhibitor, HET0016 [N-hydroxy-N-(4-butyl-2 methylphenyl)formamidine], decreases 20-HETE production, lowers blood pressure and restores endothelial function in the renal interlobar arteries of rats[30]. These data support the hypothesis of a causal role of CYP-derived eicosanoids in androgen-induced endothelial dysfunction and hypertension[30]. A more recent study, exploring the putative mechanism of hypertension in *CYP4A14* knockout mice, showed that increased oxidative stress, enhanced responses to vasoconstrictors, blunted response to vasodilators and a defect in the renal sodium excretory capacity is associated with increased 20-HETE biosynthesis[32]. Moreover, also in wild type mice, the expression of the CYP4A12, whose activity is driven by androgens, is several times higher in male with respect to female mice and correlates with the production of 20-HETE in renal microsomes[33].

The pro-hypertensive activity of 20-HETE was also tested in an experimental model of menopause: in spontaneously hypertensive female rats. Both a non-selective and a selective inhibitor of 20-HETE production reduced blood pressure in post-menopausal rats but not in fertile females and increased mRNA and protein expression of renal CYP4A isoforms was observed in postmenopausal compared to fertile rats[34].

A reduction in the synthesis of 20-HETE and EETs in the renal medulla linked to male sex has been suggested to be implicated in altered renal function and increased blood pressure in obesity-induced hypertension. In fact, in male Sprague-Dawley rats fed with high fat diet, the expression of CYP4A and CYP2C23, catalyzing the synthesis of 20-HETE and EETs, is down-regulated in renal tubules[35]. A high fat diet reduces sodium excretion and blood pressure in male rats, confirming a sex-specific effect[31]. Clofibrate and fenofibrate increase tubular CYP4A1, CYP4A8 and CYP epoxygenase expression with subsequent improvement in renal and systemic haemodynamics[36;37]. The deletion of the ephx2 gene (ephx2$^{-/-}$) in mice with subsequent increase in the EETs to diHETEs ratio was associated with transient reduction in blood pressure only in male mice[28].

Human studies exploring the patophysological role of 20-HETE and EETs in hypertension and vascular disease

Several CYP are epoxygenases and metabolize arachidonic acid to EETs in humans: CYP1A, CYP2B, CYP2E and, primarily, CYP2C and CYP2J are the subfamilies responsible for most of EETs production in cardiovascular and renal tissues[20;38;39]. As for the ω-hydroxylase activity, CYP4A and CYP4F catalyze the ω-hydroxylation of arachidonic acid to 20-HETE especially in the liver and the kidney[40]. Also sEH is expressed in several human tissues, including the kidney and the brain and may therefore be implicated in altered EETs bioactivity[41-43].

Despite abundant literature concerning the role of 20-HETE and EETs in experimental hypertension, few studies addressed the pathophysiological role of these compounds in human hypertension. Laffer and colleagues, analyzed the urinary excretion of 20-HETE in response to salt loading, furosemide administration and insulin release. Despite similar increase in the urinary excretion of 20-HETE during salt repletion, sodium excretion correlates with 20-HETE excretion in salt resistant, but not in salt sensitive subjects. This suggests that a disrupted relation between sodium and 20-HETE excretion in salt sensitive patients may results in the dependence of salt excretion on blood pressure[44]. The administration of furosemide was found associated with increased excretion of 20-HETE, correlated with changes in urinary sodium, in salt resistant and sensitive hypertensive subjects. Both sodium and 20-HETE excretion were altered in salt senisitve subjects, thus suggesting that 20-HETE modulates the natriuretic response to furosemide and that impaired natriuretic response in salt sensitive subjects implicates a mechanism controlling 20-HETE release [45]. In all these study correlation was found between urinary 20-HETE and BMI of hypertensive subect[45]. When obese hypertensive subjects were studied, not only correlation with BMI was confirmed, but also correlation between increased circulating insulin, not insulin resistance *per se*, and reduced urinary excretion of 20-HETE was found. The mechanism may implicate the inhibition of renal CYP4A by insulin, that may link obesity, high insulin levels and hypertension[46]. However, in a study including lean to overweight untreated hypertensive and normotensive subjects, despite positive correlation between urinary 20-HETE and BMI, correlation of urinary 20-HETE with serum insulin or insulin resistance was not confirmed[47]. Correlation between 20-HETE and markers of oxidative stress (F2-isoprostanes and γ-GT) as well as with 24-h diastolic blood pressure was observed in treated hypertensive subjects[48]. Interestingly, in hypertensive women, but not in men, the urinary excretion of 20-HETE was higher compared to normotensive subjects and correlated with diastolic and systolic blood pressure[49].

A limited number of studies explored the relation between CYP-derived eicosanoids and endothelial function in humans. The urinary excretion of 20-HETE was similar in non-treated hypertensive and normotensive subjects, although a negative correlation between urinary 20-HETE and endothelium-dependent vasodilation, as assessed using the method of flow-mediated dilation (FMD) of the brachial artery, has been observed[49]. The role of EETs as endothelium-derived hyperpolarizing factor (EDHF) was explored by using the CYP2C9 inhibitor sulfaphenazole. sulfaphenazole blunted the dilatory response to acetylcholine and bradykinin in the forearm microcirculation of essential hypertensive patients, while in normotensive control subjects only vasodilation residual to the effects of cyclooxygenase and nitric oxide synthase inhibitors was reduced by sulfaphenazole [50]. When urinary 20-HETE, EETs and DHETs levels were measured in plasma and urine of patients with reno-vascular disease in comparison with matched essential hypertensive and normotensive controls[51], plasma 20-HETE was found to be elevated and to correlate with plasma renin activity. This finding suggests that vasoconstrictor 20-HETE might mediate the increase in blood pressure driven by the activation of the renin-angiotensin system, typical of reno-vascular disease[51].

Genetic variations, cardiovascular diseases and sex specificity

A detailed discussion of the strength and weakness of all the studies performed to test the association between cardiovascular outcome and genetic variants in genes implicated in 20-HETE, EETs metabolism will not be discussed in details in the present review. Interest readers could refer to a recent review[52] and for the effect on coronary artery disease to a recent meta-analysis[53]. The present review will be focused on the results of a number of association studies showing an uneven sex prevalence in cerebro- and cardiovascular events, supporting the hypothesis of a sex-specific effect of altered CYPs/sEH bioactivity in hypertension. The focus has been put especially on functional SNPs. Table 1 to 4 show a summary of the results from the most studied functional SNPs, with indication of the studies identifying a sex-specific association with hypertension and cerebro- and cardiovascular diseases.

CYP2C8, CYP2C9, hypertension and cardiovascular diseases

*CYP2C8*3* (Arg139Lys and Lys399Arg, which are in 100% linkage disequilibrium; dbSNP accession number rs11572080 and rs10509681) has been shown to be associated with reduced arachidonic acid epoxygenase activity and reduced EETs production[54]. Similar observations are reported for the *CYP2C9*2* (Arg144Cys; rs1799853) and *CYP2C9*3* (Ile359Leu; rs1057910) polymorphisms.[55]

Conflicting results have been obtained from studies on *CYP2C8* polymorphisms and hypertension. Despite the evidence of decreased epoxygenases activity with consequent reduced formation of the anti-hypertensive EETs[54], no association between the *CYP2C8*3* genotype and hypertension was found in small sized studies (table 1)[56;56;57] and from a large case-control study concerning the risk of myocardial infarction in Caucasians[58]. In the latter study also the *CYP2C9*2* and *CYP2C9*3* polymorphisms were explored for a possible association with hypertension with negative results, independently of sex. A trend towards increased risk for acute myocardial infarction was found in a previous study analyzing the polymorphisms *CYP2C8*3, CYP2C9*2* and *CYP2C9*3* (table 3)[58]. In subjects enrolled in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, no significant differences in coronary artery disease between individuals carrying different *CYP2C8* and *2C9* genotypes. When the population carrying the *CYP2C9*3* allele was split according to sex, increased risk of MI was found in men and decreased risk in women[59]. The use of CYP2C9 substrates and inhibitors was associated with a significant increase in the risk of myocardial infarction especially in female harbouring a variant allele (*CYP2C8*2, CYP2C9*3)* [60]. No association between *CYP2C9*2* and *CYP2C9*3* polymorphisms and risk of subclinical atherosclerosis, ischemic vascular disease (ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease and ischemic stroke) or cardiovascular death was found in a very large Danish study[61]. Similarly, an association with coronary atherosclerosis, detected by angiography, was found in a small study performed in Turky[62]. In a mixed population from the USA, mostly of Caucasian ethnicity, no association with myocardial infarction and stroke was observed in a haplotype analysis using 8 and 7 tag SNPs for the *CYP2C8* and *CYP2C9,* respectively[63].

In conclusion, as for *CYP2C8* and *CYP2C*, limited evidence exists of a an association with increased cardiovascular risk and the presence of a sex-specific effect.

CYP2J2, hypertension and cardiovascular diseases

The *CYP2J2* -50G>T (rs890293; sometimes referred as *CYP2J2*7*) interferes with a binding site for the transcription factor Sp1 with consequent reduction in the plasma levels of EETs *in vivo*[64;65]. Carriers of the common polymorphism *CYP2J2*7* within the promoter region display reduced expression of *CYP2J2* mRNA in the heart[66]. In a Chinese population, plasma levels of 14,15-

dihydroxyeicosatrienoic acid were found significantly lower in *CYP2J2*7* T allele carriers than the in GG subjects (table 1) [67]. In a case-control study in African American, several SNPs in CYP genes, including the *CYP2J2*7,* were studied and no association was found with hypertension[57]. In a study involving 168 African American and 251 Caucasians the prevalence of the CYP2J2*7 variant allele was found significantly different among hypertensive and normotensive Caucasians but not African-Americans. Interestingly, the variant allele was found to be protective in Caucasian men, but not in women[56]. In a Han Chinese population, despite no apparent effect for the functional variant *CYP2J2**7 in the entire cohort, an association with hypertension and high systolic blood pressure was found in women homozygote for the T-allele of the intronic rs1155002 variant[68]. In more than 5,000 Swedish participants to an urban-based survey, the Malmö Diet and Cancer cardiovascular arm (MDC-CVA), no difference in the prevalence of hypertension was detected between carriers of the *CYP2J2*7* variant allele either when the entire cohort was considered or when subjects were stratified according to sex[69].

In previous case-control studies statistically significant association of the G-50T SNP was found with coronary artery disease in Germans and with premature myocardial infarction in a Taiwanese, but not in a Chinese population (table 3)[53;65;67]. Similarly, no association with cardiovascular disease was found in the aforementioned urban-based study in Swedes, in two studies including subjects at high risk for myocardial infarction and in a case-control study in Germans[69-71]. No association between the *CYP2J2*7* and coronary heart disease was observed in White Americans whereas an inverse association was evident in African Americans recruited in the ARIC study[72]. In a mixed population in the USA including mostly (90%) Caucasians, an association with myocardial infarction, but not with stroke, was found for two intronic SNPs in *CYP2J2*[63]. Also in a meta-analysis of previous studies, no association for the common CYP2J2*7 and cardiovascular disease was found[53]. The frequency of *CYP2J2*-50T allele was not significantly different in cases with ischemic stroke compared with controls in a cross-sectional study performed in a Chinese population and in a longitudinal study in Swedes[69;73]. Thus, in all the studies focusing on cerebro- and cardiovascular events, no sex-specific effects were reported or, in most cases, not even explored.

***CYP4A11*, hypertension and cardiovascular diseases**

The c.8590 T>C polymorphism (rs1126742) of the *CYP4A11* gene leads to the missense variant Phe434Ser, associated with a reduced metabolizing capacity of CYP4A11 *in vitro*[74] with consequent reduction in the urinary excretion of 20-HETE.[75] Carriers of the same polymorphism have higher aldosterone/renin and waist/hip ratios and lower furosemide-induced fractional excretion~~s~~ of sodium and potassium than *8590TT* homozygotes[76]. All these data are coherent with the selective localization of CYP4A11 in the S2 and S3 segments of proximal tubule epithelia in the cortex and outer medulla[77], so that a decreased catalytic activity should result in the reduction of 20-HETE formation and a consequent decrease in natriuresis. Therefore, the net result of having the variant allele should result in a pro-hypertensive phenotype.

Indeed, *CYP4A11* c.8590 CC-homozygotes compared to carriers of at least one “wild-type” T allele show endothelial dysfunction as demonstrated by coronary vasoconstriction induced by infusion with acetylcholine[78].

The 8590C allele of *CYP4A11*, was found associated with hypertension in the general population in Caucasian subjects from Tennessee, later replicated in the Framingham population[74], in Germans participating to the MONICA study[79] and in Swedes enrolled in the MDC-CVA study [80] (table 2). No association with hypertension was evident between the same 8590C allele and hypertension in a case control study in Australian population[75]. The 8590C allele of *CYP4A11* was associated with increased systolic blood pressure in Caucasian survivors of myocardial infarction recruited from the MONICA Augsburg myocardial infarction registry and followed up for up to 6 years[81]. In a study involving a Japanese population, the 8590C allele was associated with low risk of hypertension in the whole sample and in men[82]. In the same ethnic group, a common haplotype, containing the 8590C allele, conferred protection towards cerebral infarction (table 4) [83]. Not the functional *CYP4A11* 8590T>C but other SNPs, such as the rs9333025 and the C-296T, were found associated with cerebrovascular events in Japanese and Chinese populations, respectively[83;84]. Interestingly, the association of the rs9333025 polymorphism with cerebro-vascular events was especially evident in men[83]. In the Swedish cohort no effect of the 8590C allele of *CYP4A11* was detectable for coronary events and ischemic strokes in the whole population as well as in men and women separately[80]. In black Americans with hypertensive renal disease participating in the African American Study of Kidney Disease (AASK), men with the 8590CC genotype had significantly higher systolic blood pressure at baseline, while no association was observed in women. The same genotype was also associated with increased cumulative incidence of end stage renal disease and death[85].

CYP4F2, hypertension and cardiovascular diseases

The rs2108622 *CYP4F2* c.1347G>A (Val433Met) polymorphism causes *in vitro* a reduced arachidonic acid metabolizing capacity[86]. Consistent with this finding is the observation that carriers of the 433Met allele have decreased capacity to metabolize vitamin K[87], and tocopherol[88]. At variance with the results of these studies, the *CYP4F2* 433Met, but not the 433Val allele, increases the urinary excretion of 20-HETE in a mixed sample of hypertensive and normotensive subjects, suggesting an increased, rather than a decreased, metabolizing capacity [75]. Moreover, the increase in 20-HETE urine excretion in 433Met carriers suggests that the prohypertensive effect of the variant allele may be due to excess renal vascular production of the vasoconstrictor 20-HETE by CYP4F2. This apparently conflicts with the evidence that CYP4F2 is expressed only in the S2 and S3 segments of renal proximal tubule epithelia in the cortex and outer medulla[77].

The *CYP4F2* Val433Met genotype was not associated with increased prevalence of hypertension in the MDC-CVA, when the entire cohort was analyzed. However, an association was found in male carriers of the 433Met allele when stratifying the sample by sex (table 2) [80]. In a case-control study in Indians with stroke, an association with hypertension was found with the *CYP4F2* 433Met allele without differences between sexes[89]. In Australians the CYP4F2 GA/AA genotype (433Met allele) was significantly associated with an increase in systolic blood pressure[75] whereas in Japaneses the prevalence of the same 433Met allele was not significantly different between hypertensive cases and normotensive controls[90]. In the latter study a common haplotype and the CC genotype of the rs1558139 were associated with hypertension only in men[90]. A *CYP4F2* construct haplotype, was associated with increased excretion of 20-HETE and increased prevalence of hypertension in a case-control population study performed in China[91]. This finding was subsequently confirmed by the results of a family-based sub-analysis[91]. Notably, the *CYP4F2* 433Met allele was associated with ischemic stroke in Swedish men participating to the MDC-CVA study (table 4 and figure 1) and in a case control study in Indians[80;89]. In the latter study the association with cardioembolic stroke was strong, but no data concerning the prevalence of events in relation to sex were available. Two case-control studies were performed in Han Chinese: one showing a positive association of the 433Met allele with stroke in men [84], the second showing increased risk of ischemic stroke in male carriers of the 433Val allele. Also in a case-control study in Japanese, an association with cerebral infarction was observed in male carriers of the 433Val allele[92]. Anyhow, the sample size of the lattest studies was quite limited. In the MDC-CVA no association with coronary events was found in both sexes whereas in a case-control study performed in Han Chinese the 433Val allele was more frequently observed in cases of myocardial infarction than in controls[93].

Thus, even if a discrepancy exists in the direction of the association in people of Asian ethnicity with respect to Caucasians, a sex-specific effect is almost always present for this SNP, when explored.

Interestingly, another functional variant, c.-91T>C, included in a common haplotype (Hap I) containing SNPs in the CP4F2 regulatory region, when transfected into HEK293 cells, exhibited significantly greater LPS-stimulated activity than Hap II, that may be driven by a different NF-kappaB binding affinity between the two constructs. In vivo, a case-control study demonstrated that homozygosity for Hap I doubles the risk for hypertension in a Chinese population and is associated with higher 20-HETE excretion[91].

***EPHX2* polymorphisms and cardiovascular diseases**

In-vitro studies testing the functional capacity of 164A>G Lys55Arg (rs41507953) and the c.860G>A Arg287Gln (rs751141) polymorphisms *in EPHX2* have shown increased and diminished sEH activity, respectively[94-96], suggesting an association with lower EETs and a pro-hypertensive phenotype and higher EETs and a vasodilatory effect in the two genetic variants. In fact, a decrease in the ratio of 12,13-epoxyoctadecenoic acid to dihydroxyoctadecenoic acid was found in carriers of the *EPHX2* Lys55Arg genotype, together with higher sEH activity *ex vivo*[97]. Moreover, the *EPHX2* 287Gln variant is associated with increased neuronal survival after ischemic injury in a cell culture model[98]. Finally, the functionality of these SNPs was tested on endothelial dependent and independent vasodilatatory capacity, using intra-arterial infusion of vasoactive compounds and strain-gauge venous occlusion plethysmography. A decrease in forearm blood flow, index of impaired endothelial function, was detected in white Americans for the 55Arg variant in response to the intra-arterial infusion of bradykinin, methacholine and sodium nitroprussiate [99]. Decreased vascular resistance, suggesting a potential protective effect towards the development of hypertension, was found in Black American carriers of the *EPHX2* 287Gln variant with respect to "wild type" subjects [99].

In the MDC-CVA study a positive association of the *EPHX2* Lys55Arg variant with blood pressure/hypertension was found in men with unexpectedly high systolic blood pressure values in homozygotes with respect to carriers of at least one wild type allele[100]. In the same cohort of Swedish and in a cohort of African Americans no evidence for the *EPHX2* Arg287Gln variant influencing the development of hypertension was found[57;100].

In the Atherosclerosis Risk in Communities (ARIC) study, the *EPHX2* Lys55Arg and the Arg287Gln did not show any association with ischemic stroke, whereas two common *EPHX2* haplotypes show significant association in African-Americans and Whites respectively[101]. Different *EPHX2* haplotypes were associated with stroke also in Caucasians[102]. In the same sample, the 287Gln was associated with increased risk of cerebral ischemia, whereas no association was detectable for the Lys55Arg polymorphism[102]. Among Swedish men, those who were homozygotes for the 55Lys variant were at increased risk of ischemic stroke, while no association was evident for those carrying the Arg287Gln genotype [100]. Carriers of the *EPHX2* 287Gln allele had 50% lower risk of ischemic stroke in a Chinese population compared to 287Arg homozygotes with a positive interaction with smoke[73].

In the ARIC study, Caucasian but not African-Americans individuals harbouring the Lys55Arg polymorphism were at increased risk of coronary heart disease, with a positive association also in the analysis testing common haplotypes[97]. In the CARDIA study the Arg287Gln polymorphism, tagging a common haplotype, was associated with the presence of coronary artery calcified plaque in African Americans, while a common haplotype uniquely tagged by a polymorphism in Intron 11 was associated with significantly greater risk for coronary artery calcified plaques, in Whites[103]. In the MDC-CVA study, despite the already cited association with stroke, no association with coronary events was detected both in men and women[100]. No association was also observed in a recent study in a Chinese population and in a meta-analysis including previous studies[53].

Conclusions

Experimental models and pathophysiological studies in humans suggest that enzymes involved in 20-HETE and EETs biosynthesis and metabolism have a role in the control of blood pressure with a sex-specific effect. At least in animal models, clear evidence exists that sex hormones, especially testosterone, modulate CYPs expression and activity favouring higher blood pressure levels. Also in humans the interaction of sex hormones with CYPs expression could have a role in the well-established sexual dimorphism in blood pressure levels and cardiovascular risk described by epidemiologic studies[11;104]. In fact, SNPs in genes that cody for some of these enzymes are associated with hypertension and vascular disease, especially stroke, with notable sex-specific effect, as is the case of *CYP4F2* and *EPHX2*. Figure 2 showns the putative mechanisms by which androgens interacting with the wild type or mutant isoform of CYP4F2 could respectively increment or diminish tubular 20-HETE with subsequent modification of the individual susceptibility to hypertension and/or stroke. This model is coherent with the diminished activity of mutant CYP4F2 *in vitro,* the localization of the enzyme in the renal tubuli and his absence in the vasculature[77;105].

At the present time no specific pharmacological tools are available for studies in humans, necessary to confirm the role of CYP-derived eicosanoids in the control of blood pressure and to further investigate the proposed sex-specificity of their cardiovascular effects. Unexplored are also the potential interactions of genetic polymorphisms with environmental conditions, particularly food and dietary elements. An intriguing hypothesis to be tested is that n-3 fatty acids, acting as competitive substrates of CYPs may modulate the byosynthesis of 20-HETE and EETs and generate compunds with different vasoactive properties[106]. Future studies, in which genetic and "omic" approach are integrated with clinical and epidemiologic data, may aid to unravel the described complex tangle.

Figure legend.

Figure 1: Cumulative incidence (percentage) of ischemic strokes (left panel) and coronary events (right panel) in the Malmö Diet and cancer- cardiovascular arm according to the *CYP4F2* M433V genotype in the entire cohort (a and d), females (b and e) and males (c and f). From Fava et al *Hypertension* 2008;52(2):373-80.

Figure 2: Putative mechanisms by which androgens, interacting with the wild type or mutant isoforms of CYP4F2, may increment or diminish tubular 20-HETE biosynthesis with subsequent modification of the individual susceptibility to hypertension and/or stroke.

Table 1: Association between the most extensively studied SNPs in genes responsible for EETs formation/metabolism and hypertension in humans

Gene	SNP/SNPs (possible functionality in vitro or ex vivo)	Ethnicity	MAF %	HT /CT	Effect of the variant allele in the entire cohort	Sex-specific findings	Reference
CYP2C8	**rs11572080→*CYP2C8*3* →Arg139Lys** (n.b. associated with reduced arachidonic acid epoxygenase activity with subsequent reduced EETs production [54])	Caucasian	9.5	843/1832	No	No difference	Yasar[58]
		African American	3.3	77/75	No	N.E.	King[56]
		Caucasian	11.9	124/116	No	N.E.	King[56]
		African American	3.3	108/107	No	N.E.	Dreisbach[57]
CYP2C9	**rs1799853→CYP2C9*2→Arg144Cys** **rs1057910→CYP2C9*3→Ile359Leu** (n.b. both associated with reduced arachidonic acid epoxygenase activity with subsequent reduced EETs production[55])	Caucasian Caucasian	11.3 6.7	843/1832 843/1832	No No	No difference No difference	Yasar[58] Yasar[58]
		African American African American	3.3 2.6	108/107 108/107	No No	N.E. N.E.	Dreisbach[57] Dreisbach[57]
CYP2J2	**rs890293→ *CYP2J2*7*→-50 G>T** (n.b. associated with the interference with a binding site for the transcription factor Sp1 with consequent reduction in the plasma levels of EETs *in vivo*[64;65].	African American	10.2	102/94	No	N.E.	Dreisbach[57]
		African American	14.1	76/73	No	N.E.	King[56]
		Caucasian	7.7	123/116	Protective	Protective only in males	King[56]
		Han Chinese	2.5	415/426	No	N.E.	Wu[68]
		Russian	3.2	295/281	Deleterious	Evident in both sexes	Polonikov[107]
		Caucasian	7.8	3648/1658	No	No effect in both sexes	Fava[69]
EPHX2	**rs751141→c.860G>→Arg287Gln** (n.b. associated with diminished sEH activity [94-96])	African American	3.6	108/106	No	N.E.	Dreisbach[57]
		Caucasian	10.6	3719/2108	No	No difference	Fava[100]
EPHX2	**rs41507953→c.164A>G→Lys55Arg;** (n.b. associated with diminished sEH activity [94-96])	Caucasian	9.2	3746/2129	No	Evident in males but not females	Fava[100]

HT: hypertension; CT: either control or normotensive subjects; N.E. non explored; BP, blood pressure

Table 2: Association between the most extensively studied SNPs in genes responsible for 20-HETE formation and hypertension in humans

Gene	SNP/SNPs	Ethnicity	MAF %	HT /CT	Effect of the variant allele in the entire cohort	Sex-specific findings	Reference
CYP4A11	**rs1126742➔c.8590 T>C ➔ Phe434Ser** (n.b. associated with reduced metabolizing capacity of CYP4A11 *in vitro*[74] with consequent reduction in the urinary excretion of 20-HETE.[75]**)**	African-American	30.0	60/60	No effect	No difference	Gainer[74]
		White American	15.7	197/195	Deleterious	No difference	Gainer[74]
		White American	12.5	868/670	Deleterious	No difference	Gainer[74]
		Caucasian	13.3	649/748	Higher prevalence of HT	No difference	Meyer[79]
		Caucasian	15.1	228/332	Higher SBP in survivors of MI	N.E.	Meyer[81]
		Australian	15.3	161/74	None for HT and BP	No difference	Ward[75]
		Caucasian	12.5	3805/2170	Higher BP and HT prevalence in CC-HZ	No differences	Fava[80]
		Japanese	20.6	304/207	Protective	Evident in males but not females	Fu[82]
		African American	30.9	732	Higher SBP in CC-HZ	Evaluated only in men	Gainer[85]
CYP4F2	**rs2108622➔c.1347G>A➔Val433Met** (n.b. associated with a reduced arachidonic acid metabolizing capacity[86] but contrarily to what could be expected with increased urinary excretion of 20-HETE[75]**)**	Australian	28.7	161/74	None on HT and BP	Higher SBP in female A-carriers	Ward[75]
		Caucasian	26.1	3700/2092	No effect	Higher BP and HT only in males	Fava[80]
		Japanase	27.4	249/238	No effect	No differences	Fu[90]
		Indians	50	427/567	Higher prevalence of HT	N.E.	Munshi[89]

HT: hypertension; CT: either control or normotensive subjects; N.E. non explored; BP, blood pressure; HZ, Homozygotes

Table 3: Association between the most extensively studied SNPs in genes responsible for EETs formation/metabolism and cardiovascular diseases in humans

Gene	SNP/SNPs	Ethnicity	MAF %	Cases /CT	Effect of the variant allele in the entire cohort	Sex-specific findings	Reference
CYP2C8	**rs11572080➔*CYP2C8*3*➔Arg139Lys**	African American	1.9	224/309	No for CHD	N.E.	Lee[72]
		Caucasian	10.9	773/577	No for CHD	N.E.	Lee[72]
		Caucasian	9.5	1172/1503	Trend vs. higher prevalence in MI cases	No difference	Yasar[58]
		Caucasian	10.1	1052/615	No difference	No difference	Haschke-Becher[59]
CYP2C9	**rs1799853➔CYP2C9*2➔Arg144Cys** **rs1057910➔CYP2C9*3➔Ile359Leu**	Caucasian	11.3 6.7	1172/1503	Trend vs. higher prevalence in MI cases	No difference	Yasar[58]
		Caucasian	11.4 6.9	1052/615	No difference in MI and CAD	Men and women carrying the CYP2C9*3 allele had respectively an increased and decreased risk of IHD	Haschke-Becher[59]
		Caucasian	12.8 5.8	223/5753	No difference	Higher risk in women with a variant allele	Visser[60]
		Caucasian	34 carriers*	9469/51898	No difference for IHD, MI	N.E.	Kaur-Knudsen[61]
		Turkish	10.9 17.7	108/90	No difference in CAD	N.E.	Ercan[62]
CYP2J2	**rs890293➔ *CYP2J2*7*➔-50 G>T**	Caucasian	7.6	289/255	Higher prevalence in CAD	N.E.	Spiecker[65]
		Taiwanese	15	200/200	Higher prevalence in smokers with premature MI	N.E.	Liu[67]
		Caucasian	6.4	2547/697	No association with CAD, MI and death	N.E.	Hoffmann[71]
		Han Chinese	6.6	1344/1267	No	N.E.	Xu[53]
		African American	15.5	224/309	lower risk of incident CHD events	N.E.	Lee[72]
		Caucasian	6.4	773/577	No for CHD	N.E.	Lee[72]
		Caucasian	7.7	146/854	No effect	N.E.	Borgel[70]
		Caucasian	7.8	261/5478	No effect for incident	No effect in both sexes	Fava[69]

					coronary events		
		Caucasian	7.8	185/5554	No effect for incident strokes	No effect in both sexes	Fava[69]
		Chinese	4.7	200/350	No effect for incident strokes	N.E.	Zhang[73]
EPHX2	**rs751141➔c.860G>A➔Arg287Gln**	African American Caucasian	8.6 10.4	315/1021	No effect for ischemic stroke	N.E.	Fornage[101]
		African American Caucasian	8.0 10.6	1086/980	No effect for CHD	No difference	Lee[97]
		Caucasian	10.6	274/5560	No difference for coronary events	No difference	Fava[100]
		Caucasian	10.6	197/5560	No difference for stroke	No difference	Fava[100]
		Han Chinese	22.3	1344/1267	No	N.E.	Xu[53]
		Chinese	19.9	200/350	Lower prevalence in stroke patients	N.E.	Zhang[73]
		Caucasian	9.8	601/736	Higher prevalence of the variant allele in IS	N.E.	Gschwendtner[102]
		African American Whites	8.8 10.6	286/2696	Higher prevalence in AA with CAC	N.E.	Wei[103]
EPHX2	**rs41507953➔c.164A>G➔Lys55Arg;**	Caucasian	9.2	274/5560	No difference for coronary events	No difference	Fava[100]
		Caucasian	9.2	197/5560	No difference for stroke	Evident in males but not females	Fava[100]
		African American Caucasian	22.3 9.8	1086/980	Higher prevalence of the variant allele in Caucasian	No difference	Lee[97]
		African American Caucasian	22.8 10.2	315/1021	No effect for ischemic stroke	N.E.	Fornage[101]
		African American Whites	22.1 11.3	286/2696	No difference in CAC	N.E.	Wei[103]
		Caucasian	9.1	601/736	No effect for ischemic stroke	N.E.	Gschwendtner[102]

N.E., non explored; CAC, coronary artery calcified plaques; CAD, coronary artery disease; CAD, coronary heart disease; IHD, ischemic heart disease; MI, myocardial infarction; IS, ischemic stroke

Table 4: Association between the most extensively studied SNPs in genes responsible for 20-HETE formation and cardiovascular diseases in humans

Gene	SNP/SNPs	Ethnicity	MAF %	Cases /CT	Effect of the variant allele in the entire cohort	Sex-specific findings	Reference
CYP4A11	**rs1126742➔c.8590 T>C➔Phe434Ser**	Caucasian	12.5	276/5554	No difference in coronary events	No differences	Fava[80]
		Caucasian	12.5	199/5554	No difference in stroke	No differences	Fava[80]
		Japanese	20.5	174/293	No effect for cerebral infarction	No differences	Fu[83]
		Chinese	20.0	779/557	No effect for ischemic stroke	No differences	Ding[84]
CYP4F2	**rs2108622➔c.1347G>A➔Val433Met**	Japanase	28.7	175/246	No effect for cerebral infarction	The G allele is associated with cerebral infarction in men	Fu[92]
		Japanase	28.7	234/248	No effect for MI	The G allele is associated with MI in men	Fu[93]
		Indians	50	507/487	Higher prevalence in stroke	N.E.	Munshi[89]
		Caucasian	26.1	276/5554	No effect for coronary events	No differences	Fava[80]
		Caucasian	26.1	199/5554	No effect for strokes	Higher prevalence in men with stroke	Fava[80]
		Chinese	22.9	779/557	Associated with ischemic stroke	Evident only in men	Ding[84]
		Han Chinese	29.1	302/350	No effect for stroke	The wild type was associated with stroke only in men	Deng[108]

N.E., non explored; CAC, coronary artery calcified plaques; CAD, coronary artery disease; CAD, coronary heart disease; IHD, ischemic heart disease; MI, myocardial infarction; IS, ischemic stroke

Figure 1

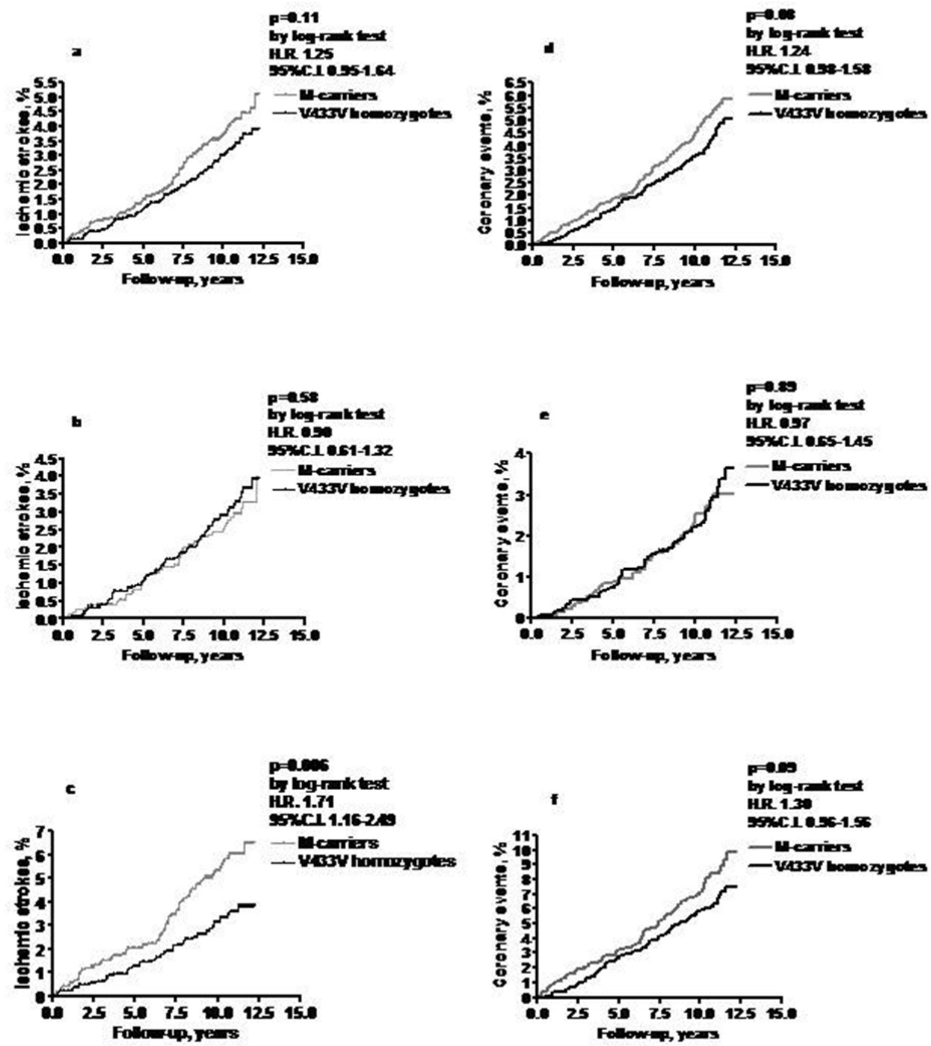
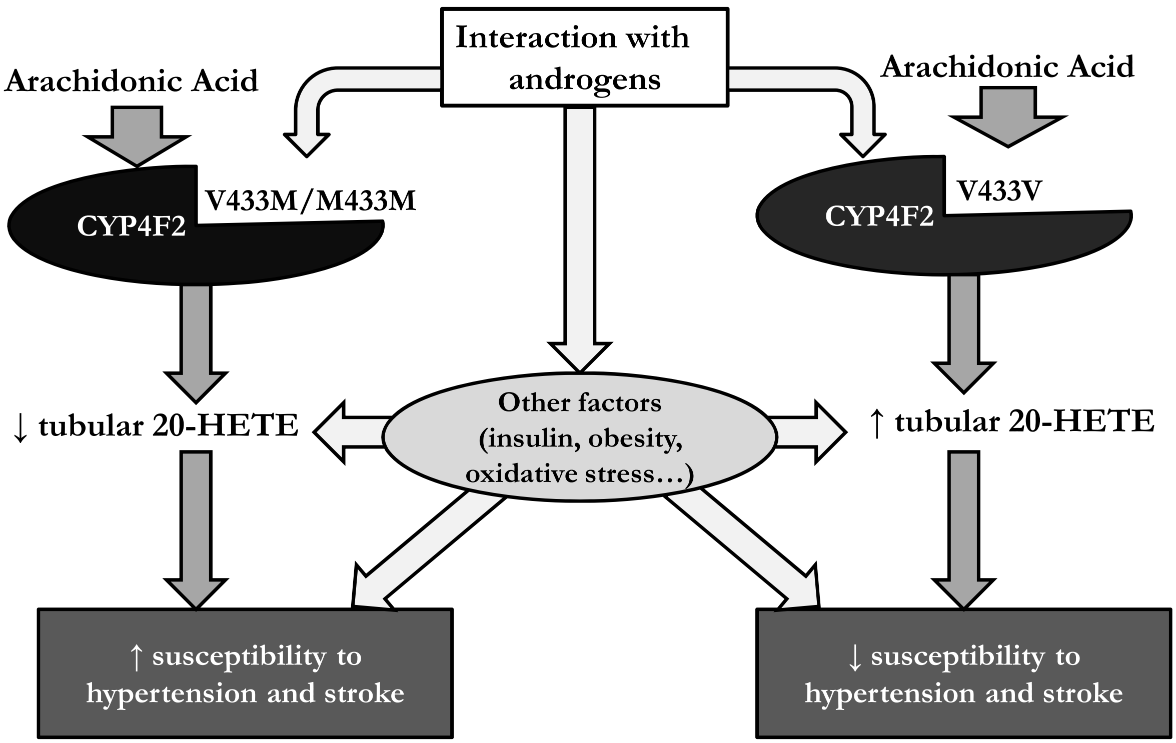

a
p=0.11
by log-rank test
H.R. 1.25
95%C.I. 0.95-1.64
M-carriers
V433V homozygotes
Ischemic strokes, %
Follow-up, years
d
p=0.08
by log-rank test
H.R. 1.24
95%C.I. 0.98-1.58
M-carriers
V433V homozygotes
Coronary events, %
Follow-up, years
b
p=0.58
by log-rank test
H.R. 0.90
95%C.I. 0.61-1.32
M-carriers
V433V homozygotes
Ischemic strokes, %
Follow-up, years
e
p=0.89
by log-rank test
H.R. 0.97
95%C.I. 0.65-1.45
M-carriers
V433V homozygotes
Coronary events, %
Follow-up, years
c
p=0.006
by log-rank test
H.R. 1.71
95%C.I. 1.16-2.49
M-carriers
V433V homozygotes
Ischemic strokes, %
Follow-up, years
f
p=0.09
by log-rank test
H.R. 1.30
95%C.I. 0.96-1.56
M-carriers
V433V homozygotes
Coronary events, %
Follow-up, years

Figure 2



Interaction with androgens
Arachidonic Acid
CYP4F2
V433M/M433M
Arachidonic Acid
CYP4F2
V433V
↓ tubular 20-HETE
Other factors (insulin, obesity, oxidative stress…)
↑ tubular 20-HETE
↑ susceptibility to hypertension and stroke
↓ susceptibility to hypertension and stroke

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